Product information

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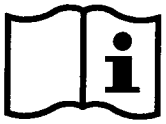

User's Manual

ENA-6 Profile ELISA

Enzyme immunoassay for the qualitative measurement of IgG class autoantibodies directed against extractable nuclear antigens in human serum or plasma.

CE

IVD

REF **DE7410**

Σ **96 wells**

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1. INTENDED PURPOSE

ENA-6-Profile is an ELISA test system for the qualitative measurement of IgG class autoantibodies directed against the extractable nuclear antigens SS-A (52 and 60 kDa, Ro), SS-B (La), Sm, RNP/Sm, Scl-70, and Jo-1 in human serum or plasma. This product is intended for professional in vitro diagnostic use only.
The test is used as an aid in the differential diagnosis of inflammatory autoimmune diseases, e.g. systemic lupus erythematosus, mixed connective tissue disease, Sjoegren's syndrome, scleroderma, and polymyositis/dermatomyositis. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

2. PRINCIPLE OF THE TEST

Highly purified antigens SS-A (52 and 60 kDa), SS-B, Sm, RNP/Sm, Scl-70 and Jo-1 are bound to microwells. Antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

3. WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
- Calibrators, Controls, sample buffer and Wash Solution contain sodium azide (NaN_3) 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitrile rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

4. CONTENTS OF THE KIT

Sufficient for 96 determinations

SORB MT 1 divisible microplate consisting of 12 modules of 8 wells each. Ready to use.

CAL SS-A 1x 1 ml Calibrator SS-A (cut-off), containing SS-A antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.

CAL SS-B 1x 1 ml Calibrator SS-B (cut-off), containing SS-B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.

CAL Sm 1x 1 ml Calibrator Sm (cut-off), containing Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.

CAL RNP/Sm 1x 1 ml Calibrator RNP/Sm (cut-off), containing RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.

CAL Scl-70 1x 1 ml Calibrator Scl-70 (cut-off), containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.

CAL Jo-1 1x 1 ml Calibrator Jo-1 (cut-off), containing Jo-1 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.

CONTROL 1 & 2 2x 2 ml Control positive (1) and negative (2), containing ENA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.

SAM DIL 5x 20 ml Sample Buffer, containing PBS, BSA, detergent, preservative NaN_3 0.09%, yellow, 5x conc.

ENZ CONJ 15 ml Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use.

SUB TMB 15 ml TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.

STOP SOLN 15 ml Stop solution; contains acid. Ready to use.

WASH SOLN 50x 20 ml Wash Buffer, containing Tris, detergent, preservative NaN_3 0.09%;

50 x conc.

1 Instruction for Use

1 Certificate of Analysis

5. MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

6. SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

7. STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and desiccated in the clip bag provided.
- Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Solution and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

8. PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, perform the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of Wash Solution.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

9. PREPARATION OF REAGENTS

Wash Buffer
Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

Sample Buffer
Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples
Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

10. TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette 100 µl of calibrators, controls and prediluted patient samples into the wells.
2. Incubate for 30 minutes at room temperature (20-28 °C).
3. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
4. Dispense 100 µl of enzyme conjugate into each well.
5. Incubate for 15 minutes at room temperature.
6. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
7. Dispense 100 µl of TMB substrate solution into each well.
8. Incubate for 15 minutes at room temperature
9. Add 100 µl of stop solution to each well of the modules
10. Incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm (reference 600-690nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	C+	C+	C+	C+	C+	C+						
B	**SS-A**	**SS-B**	**Sm**	**RNP**	**Scl**	**Jo-1**						
C	C-	C-	C-	C-	C-	C-						
D	P1	P1	P1	P1	P1	P1						
E	P2	P2	P2	P2	P2	P2						
F	P3	P3	P3	P3	P3	P3						
G	P4	P4	P4	P4	P4	P4						
H	P5	P5	P5	P5	P5	P5						
	red	blue	yellow	green	pink	black						

Antigen coated in the modules:
Red = SS-A
Blue = SS-B
Yellow = Sm
Green = RNP
Pink = Scl-70
Black = Jo-1

P1,…patient samples; Specific calibrators: **SS-A**, **SS-B**, **Sm**, **RNP**, **Scl**, **Jo-1**; C+,C-: controls

11. VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit. If these quality control criteria are not met the assay run is invalid and should be repeated.

12. CALCULATION OF RESULTS

For qualitative results: for each antigen of the profile the optical density (OD) of a sample is compared to the OD of the antigen-specific Calibrator

Negative: OD sample < OD Calibrator
Positive: OD sample ≥ OD Calibrator

For detailed results the optical density of a sample is expressed as Index value:

Index = OD sample / OD Calibrator

13. PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the internationally recognized reference sera from CDC, Atlanta USA.

Measuring range

not applicable

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off Index 1.0

Interpretation of results

Negative: Index < 1.0 Borderline: Index 1.0 - 1.2 Positive: Index > 1.2

Linearity
Patient samples containing high levels of specific antibody were serially diluted in sample buffer. Activity for each dilution step was calculated as Index- Value.

Sample	Dilution	Observed Index	Expected Index	O/E (%)
SS-A	1:100	4.9	4.9	100
	1:200	2.5	2.5	103
	1:400	1.3	1.2	105
	1:800	0.6	0.6	98
SS-B	1:100	5.2	5.2	100
	1:200	2.5	2.6	97
	1:400	1.3	1.3	95
	1:800	0.6	0.7	88
Sm	1:100	4.5	4.5	100
	1:200	2.2	2.3	96
	1:400	1.1	1.1	96
	1:800	0.5	0.6	89
RNP/Sm	1:100	4.2	4.2	100
	1:200	2.0	2.1	96
	1:400	1.0	1.0	94
	1:800	0.5	0.5	94
Scl-70	1:100	4.0	4.0	100
	1:200	1.9	2.0	93
	1:400	1.0	1.0	102
	1:800	0.5	0.5	98
Jo-1	1:100	4.3	4.3	100
	1:200	2.1	2.2	96
	1:400	1.0	1.1	96
	1:800	0.5	0.5	94

Limit of detection
not applicable

Reproducibility
Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	**Mean Index**	**CV %**
SS-A	1.5	4.3
SS-B	1.3	5.0
Sm	1.7	3.1
RNP/Sm	1.1	3.9
Scl-70	1.3	3.1
Jo-1	1.4	4.2

Inter-Assay		
Sample	**Mean Index**	**CV %**
SS-A	1.4	3.6
SS-B	1.2	4.0
Sm	1.7	2.3
RNP/Sm	1.2	3.6
Scl-70	1.2	2.9
Jo-1	1.3	3.4

Interfering substances
No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparin). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos*	%
SLE	63	56	88.9
Sjörgen's Disease	10	10	100.0
MCTD	10	10	100.0
Scleroderma	10	3	30.0
Poly-Dermatomyositis	8	5	62.5
CREST Syndrome	9	4	44.4
Rheumatoid arthritis	60	2	3.3
Normal human sera	148	3	2.0

*positive for one or more antigens

Clinical Diagnosis

	Pos	Neg	
Pos	95	5	
Neg	15	203	
	110	208	318

Sensitivity: 86.4 %
Specificity: 97.6 %
Overall agreement: 93.7 %

14. LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually. The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

15. REFERENCES

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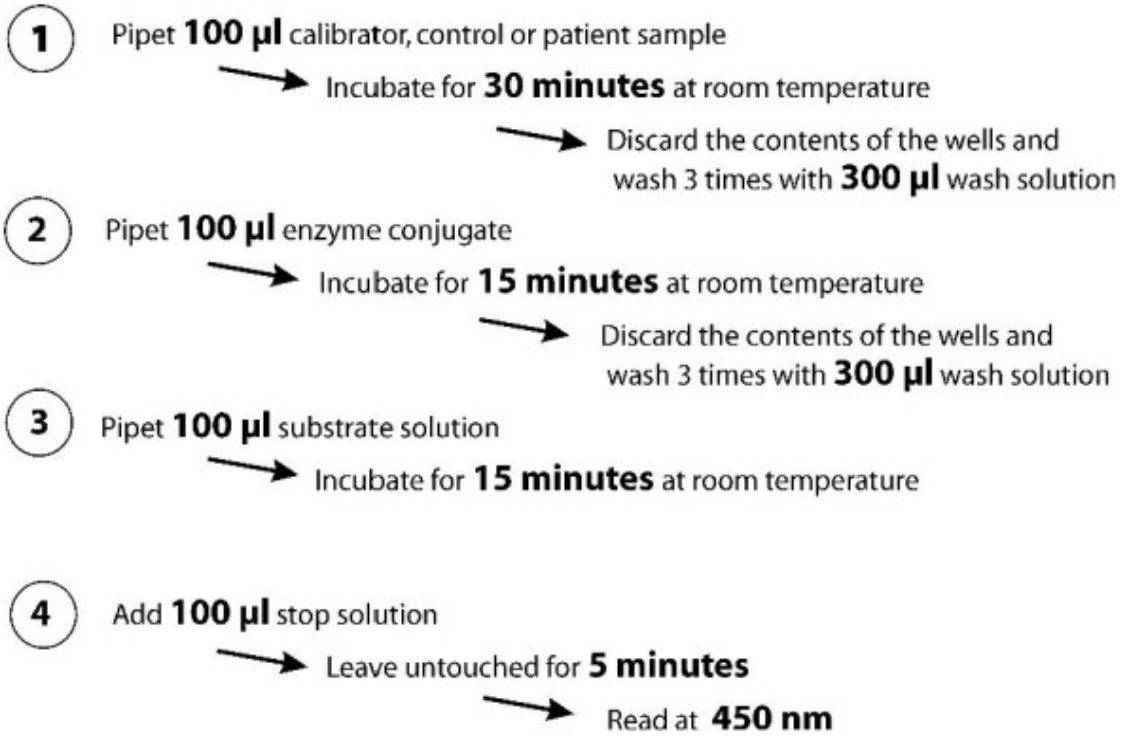

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore